See related Commentary on page ix

High Ultraviolet A Protection Affords Greater Immune Protection Confirming that Ultraviolet A Contributes to Photoimmunosuppression in Humans

Elma D. Baron,* Anny Fourtanier,† Delphine Compan,† Chantal Medaisko,† Kevin D. Cooper,* and Seth R. Stevens*

*Department of Dermatology, University Hospitals of Cleveland/Case Western Reserve University, Cleveland, Ohio, USA; and †L' Oréal Recherche, Clichy, France

Solar radiation causes immunosuppression that contributes to skin cancer growth. Photoprotective strategies initially focused on the more erythemogenic ultraviolet B. More recently, the relationship of ultraviolet A and skin cancer has received increased attention. We hypothesized that if ultraviolet A contributes significantly to human ultraviolet-induced immune suppression, then increased ultraviolet A filtration by a sunscreen would better protect the immune system during ultraviolet exposure. Two hundred and eleven volunteers were randomized into study groups and received solar-simulated radiation, ranging from 0 to 2 minimum erythema dose, on gluteal skin, with or without sunscreen, 48 h prior to sensitization with dinitrochlorobenzene. Contact hypersensitivity response was evaluated by measuring the increase in skin fold thickness of five graded dinitrochlorobenzene challenge sites on the arm, 2 wk after sensitization. Clinical scoring using the North American Contact Dermatitis Group method was also performed. Solar-simulated radiation dose–response curves were generated and immune protection factor was calculated using a nonlinear regression model. Significance of immune protection between study groups was determined with the Mann–Whitney–Wilcoxon exact test. The sunscreen with high ultraviolet A absorption (ultraviolet A protection factor of 10, based on the *in vivo* persistent pigment darkening method) and a labeled sun protection factor of 15 demonstrated better immune protection than the product that had a low ultraviolet A absorption (ultraviolet A protection factor of 2) and a labeled sun protection factor of 15. Nonlinear regression analysis based on skin fold thickness increase revealed that the high ultraviolet A protection factor sunscreen had an immune protection factor of 50, more than three times its sun protection factor, whereas the low ultraviolet A protection factor sunscreen had an immune protection factor of 15, which was equal to its labeled sun protection factor. This study demonstrates that ultraviolet A contributes greatly to human immune suppression and that a broad-spectrum sunscreen with high ultraviolet A filtering capacity results in immune protection that exceeds erythema protection. These results show that high ultraviolet A protection is required to protect against ultraviolet-induced damage to cutaneous immunity. ***Key words: contact hypersensitivity/immune protection/sunscreen/ultraviolet light. J Invest Dermatol 121:869–875, 2003***

Suppression of the skin's immune system is known to be one of the mechanisms by which solar ultraviolet (UV) radiation induces skin cancer growth (Ullrich, 2002). Sunscreens have been shown to afford protection against UV-induced immune suppression, although to date the degree of immune protection afforded by these products falls short of the degree to which they prevent erythema (Ullrich *et al*, 1999; Kelly *et al*, 2003). Because immune suppression occurs at suberythemogenic doses (Cooper *et al*, 1992; Kelly *et al*, 2000), this level of immune protection is likely to be inadequate. Most of these studies, however, were conducted using sunscreens that filtered mainly UVB (290–320 nm) and, to some extent, UVAII (320–340 nm). Recently, there has been a greater awareness regarding the immunosuppressive role of UVA (320–400 nm) (Bestak and Halliday, 1996; LeVee *et al*, 1997; Damian *et al*, 1999; Dumay *et al*, 2001; Nghiem *et al*, 2001). For example, Nghiem *et al* (2001) demonstrated in mice that UVA effectively suppresses the elicitation of an established immune response to *Candida albicans*. Kuchel *et al* (2002) showed that additional UVA augments solar-simulated radiation (SSR) induced suppression of elicitation responses to nickel in nickel-sensitive individuals. LeVee *et al* (1997) showed that UVAII (320–340 nm) may have suppressive effects on the induction of contact sensitization. Other work focusing on the benefit of broad-spectrum coverage (Bestak *et al*, 1995; Damian *et al*, 1997; Moyal *et al*, 1997; Molen *et al*, 2000; Moyal and Fourtanier, 2001) and the need for determining a product's level of protection in the UVA range (UVA-PF)

Manuscript received January 31, 2003; revised April 16, 2003; accepted for publication April 23, 2003

Reprint requests to: Elma D. Baron, MD, Department of Dermatology, University Hospitals of Cleveland, 11100 Euclid Avenue, Lakeside Bldg 3500, Cleveland, OH 44106. Email: edb4@po.cwru.edu

Abbreviations: CHS, contact hypersensitivity; IPF, immune protection factor; PPD, persistant pigament darkening; SFT, skin fold thickness; SPF, sun protection factor; SSR, solar-simulated radiation; UVA-PF, protection factor in the UVA range.

0022-202X/03/$15.00 ·

(Fourtanier *et al*, 2000; Gasparro, 2000; Lim *et al*, 2000; Nghiem *et al*, 2001; Baron and Stevens, 2002) likewise alludes to the contributory role of UVA in photoimmune suppression. Nonetheless, the actual effect of UVA protection level on the capacity of a sunscreen formula to prevent immune suppression has not been demonstrated in human studies.

This study was performed to determine whether increased delivery of UVA accompanying SSR would increase immune suppression and, conversely, whether added protection against UVA (or high UVA absorption) would increase the immune protection afforded by sunscreens. This was done by evaluating the efficacy of two commercial sun protection factor (SPF) 15 broad-spectrum sunscreens in preventing UV-induced local suppression of contact hypersensitivity (CHS) response to dinitrochlorobenzene (DNCB) in human subjects.

MATERIALS AND METHODS

All procedures were approved by the Institutional Review Board, University Hospitals of Cleveland Research Institute/Case Western Reserve University.

Subjects Healthy individuals between 18 and 60 y of age, with Fitzpatrick skin types I–IV, were recruited. Written informed consent was obtained. Excluded were those on systemic medication (except contraceptive pills) and those with significant medical and/or dermatologic history or photosensitivity. The minimum erythema dose (MED) of each subject was determined and those with an MED of 20–50 mJ per cm^2 of UVB were enrolled. This is equivalent to about 2–7 J per cm^2 of total UV dose (i.e., UVA + UVB) from the full spectrum of SSR. Each qualified subject was then randomized to a study group.

UV light source SSR was delivered using a 1000 W xenon arc solar simulator model 6271 (Oriel Instruments, Stratford, CT), with a dichroic mirror and 81017bis filter (WG320/1.5 mm), producing a spectrum of 290–400 nm. This spectrum as well as the integrated irradiance were measured with a calibrated Bentham DM 150 double monochromator spectroradiometer. Irradiance was measured routinely using an IL1700 radiometer (International Light, Newburyport, MA) equipped with a sensor for UVA (SED 033, UVA filter 19672) and UVB (SED 240, UVB filter 15541) positioned 10 inches from the light source.

Sunscreen products The two sunscreens are commercial US formulations with a labeled SPF of 15.

Absorption spectra of both products were generated by spectroradiometric measurements between 290 and 400 nm according to a modified Diffey method (Diffey and Robson, 1989) (**Fig 1**).

The critical wavelength (λ_c) was measured according to the Diffey method (Diffey *et al*, 2000). A λ_c value superior to 370 nm is a criterion for a broad-spectrum claim. The SPF values were checked using FDA standard recommendations for SPF determination (Federal Register, 1999) on 10 volunteers not included in the CHS study. UVA-PF was determined on 10 other subjects using an *in vivo* method based on persistent pigment darkening dose (Moyal *et al*, 2000).

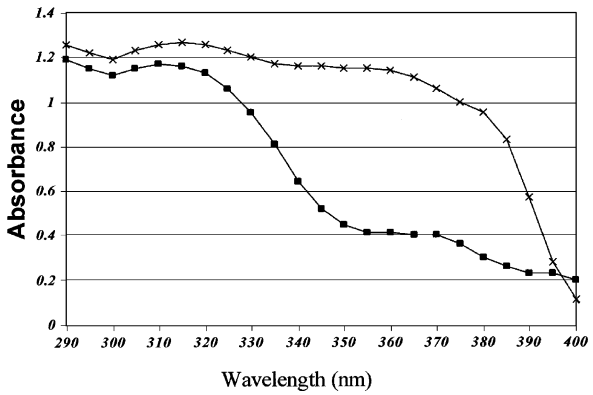

Figure 1. Comparison of the absorption spectra of the two sunscreen products via spectroradiometric measurements between 290 and 400 nm. The spectral curve of product A (high UVA absorption sunscreen) is higher than that of product B.

Table I. Properties of two commercial SPF 15 sunscreens

	Product A	Product B	
Label SPF	15	15	
Actual back US SPF (mm ± SD)	18.78 ± 3.79	15.49 ± 3.91	Not significant
	$n = 10$	$n = 10$	
UVA-PF *in vivo* PPD (mm ± SD)	10.4 ± 1.4	2.4 ± 0.4	$p < 0.01$
λ_c (critical wavelength)	380 nm	372 nm	

The high UVA absorption sunscreen (product A) contains avobenzone (Parsol 1789), octocrylene (Uvinul N 539), and octyl salicylate. The low UVA absorption sunscreen (product B) contains zinc oxide and octyl methoxycinnamate (Parsol MCX) (**Table I**).

MED determination The MED was determined by exposing eight 1 cm areas of gluteal skin to increasing doses of SSR from approximately 1 to 8 J per cm^2 of total UV dose. Erythema was assessed 16–24 h later, both by visual evaluation and by colorimetric measurement using a chromometer (CR-300 Minolta, Tokyo, Japan). Linear regression was applied and 1 MED was calculated according to COLIPA recommendations (Anonymous, 1996) as the dose of UV producing an increase in the redness parameter (δ_a) of + 2.5.

SPF testing The standard SPF test procedure (COLIPA method) (Anonymous, 1996) was performed for subjects assigned to study groups that would undergo sunscreen-protected SSR irradiation. Briefly, product was applied over a 48 cm^2 area of the buttock at a dose of 2 mg per cm^2. After 15 min, six 1 cm^2 areas were then exposed to increasing doses of SSR ranging from approximately 30 to 150 J per cm^2 of total UV. A standard MED test was simultaneously performed on the unprotected contralateral gluteal area for comparison. Visual and colorimetric MED readings were performed 16–24 h postirradiation. SPF was calculated by dividing the sunscreen-protected MED by the unprotected MED.

SSR irradiation protocol SSR was delivered over a 1 inch square area of gluteal skin. For product A, five groups of subjects were given unprotected SSR exposures at doses of 0, 0.25, 0.5, 0.75, and 1.0 times their baseline MED, respectively, whereas seven groups underwent sunscreen application as described above, followed 15 min later by SSR irradiation at doses of 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 MED, multiplied by the specific SPF value obtained from the individual. For product B, three groups were given unprotected SSR at 0, 0.5, and 0.75 MED, whereas four groups were given protected SSR exposures at 0, 0.5, 0.75, and 1.0 MED multiplied by the individual SPF.

DNCB sensitization Sensitization with DNCB was performed on the SSR-irradiated site 2 d after exposure. The area to be sensitized was first evaluated for erythema both visually and by colorimetry. A 48 μL acetone solution of 0.0625% DNCB (30 μg DNCB) was then applied on the skin using a filter-paper-lined 1.2 cm Finn chamber, and was kept in place for 48 h.

DNCB challenge Two weeks after sensitization, DNCB challenge was performed on the contralateral upper inner arm. Twenty microliter solutions of 0, 3.125, 6.25, 8.75, and 12.5 μg DNCB were applied via five filter-paper-backed 8 mm Finn chambers that were kept in place for 6 h. The skin fold thickness (SFT) of the five challenge sites was measured before application of the patches and 48 h later using a micrometer (Mitutoyo, Japan). The total increase in SFT (in millimeters) from the five challenge sites was then determined per subject. A clinical score based on the North American Contact Dermatitis Group (NACDG) system was also recorded for each challenge site as follows: 1, no reaction; 2, macular erythema; 3, erythema with induration; 4, vesicular/blistering reaction. The total score from the five challenge sites was then calculated for each subject. These two parameters represent the CHS response for each volunteer. Immune suppression occurs if the immune response observed in an exposed group is significantly lower than that of the untreated unexposed sensitized group.

Data analysis Comparisons between groups were performed by exact Mann–Whitney–Wilcoxon tests, at a two-tailed 5% significance level. To determine each product's immune protection factor (IPF), individual CHS responses, expressed as (1) total millimeter increase in SFT and (2) total NACDG score, were plotted against total UV dose delivered in joules per

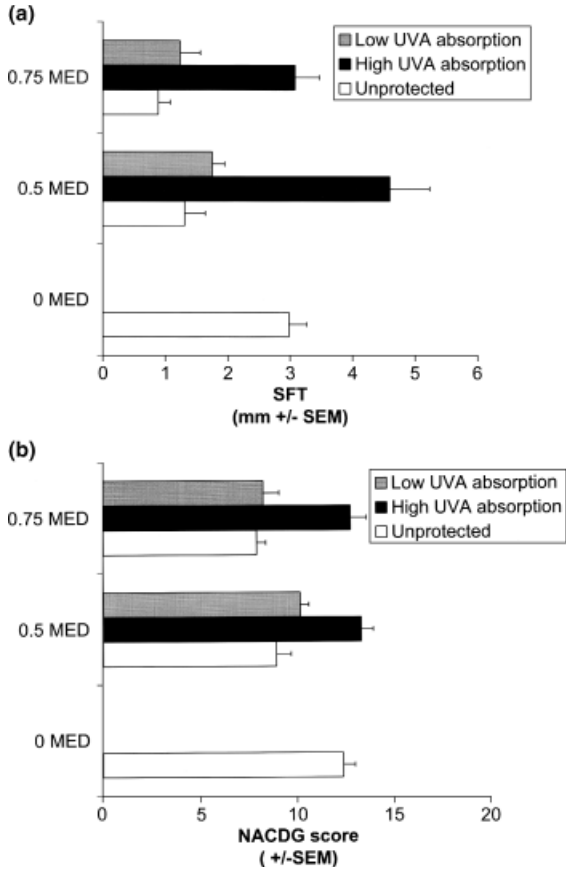

Figure 2. Characteristics of sunscreens tested. Mean CHS response at 0.5 and 0.75 MED doses were compared for unprotected, high UVA absorption sunscreen-protected, and low UVA absorption sunscreen-protected groups, based on SFT increase (*a*) and NACDG score (*b*). Using exact Mann–Whitney–Wilcoxon tests, significant CHS suppression was not obtained at either dose among groups protected by the high UVA absorption sunscreen. Significant CHS suppression was observed in the groups that used a low UVA absorption sunscreen starting at 0.5 MED.

square centimeter. Nonlinear regressions were generated from the different UV dose–response curves based on the following two-parameter exponential model:

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where γ is response (SFT or NACDG score) and trt equals 0 for unprotected groups and 1 for sunscreen-treated groups. Associated estimates for IPF are given with their 95% bilateral confidence intervals (**Figs 3, 4**). These IPFs are global protection levels, which are not based on a specific level of biologic response (e.g., minimal or 50% maximal) but instead are a measure of protection across the entire UV dose–response range. All analyses were performed on SAS release 8.2 and SPSS release 9.0 statistical software.

RESULTS

Patient characteristics and SPF results A total of 211 volunteers, 85 males and 126 females, with a mean age of 27 y (range 18–59), completed the study. The Fitzpatrick phototype (Freedberg *et al*, 1997) distribution was as follows: I, 18; II, 133; III, 58; IV, 2; the phototypes were fairly distributed among study groups. Subjects who were randomized into groups receiving sunscreen-protected SSR exposure underwent SPF determination. This revealed an average SPF value of 15.4 ± 3.4 SD (range 7.6–23.6) for product A ($n = 57$) and 9.9 ± 2.3 SD (range 6.0–15.7) for product B ($n = 30$). The wide range of values obtained highlights the need for testing individual SPF.

Irritancy and positive controls To test the irritancy component of the allergen DNCB, a total of 18 volunteers underwent DNCB elicitation on the arm without prior sensitization. This yielded a mean SFT increase, in millimeters, of 0.31 ± 0.19 SD and a mean clinical score of 5.67 ± 1.03 SD, based on the NACDG system. In contrast, a total of 21 subjects who underwent DNCB sensitization on the buttock 2 wk prior to DNCB elicitation on the arm demonstrated a mean SFT increase of 2.98 ± 1.32 SD and a mean NACDG score of 12.38 ± 2.84 SD. Statistical analysis via exact Mann–Whitney–Wilcoxon test revealed a significant difference between the irritancy and positive control groups based on both SFT and NACDG score ($p < 0.01$).

Higher degree of immune suppression was obtained with increased UVA exposure The immune protection afforded by each product was compared at two suberythemogenic doses (0.5 and 0.75 MED) (**Fig 2**). Results indicate that the product with high UVA absorption afforded a significantly higher degree of immune protection than the product with low UVA absorption. Using Mann–Whitney–Wilcoxon exact tests, significant immune suppression among unprotected subjects was observed for both endpoints (SFT and NACDG) at 0.5 MED ($p = 0.001$). Whereas subjects who underwent SSR exposure after applying the low UVA absorption sunscreen demonstrated significant immune suppression at the 0.5 MED $\times$ SPF dose and the 0.75 MED $\times$ SPF dose ($p < 0.05$), those who received less UVA irradiation by using the sunscreen with high UVA absorption did not demonstrate significant immune suppression at these two doses ($p > 0.4$) and even at the dose of 1 MED $\times$ SPF ($p > 0.1$) for SFT.

Although it may seem surprising that the mean CHS response of the product-A-protected subjects in the 0.5 MED group exceeded that of the unirradiated controls, this may be explained by the fact that the mean is sometimes tilted in favor of extremely high values such as the SFT readings in millimeters obtained from some subjects who had very strong reactions. This disparity in CHS response was observed less when clinical scoring was used because there is an upper limit of 4 in the NACDG scale.

High UVA filtration protects against immune suppression more than erythema To calculate the sunscreen's level of efficacy against immune suppression (i.e., IPF), CHS responses based on SFT increase and clinical score were plotted against total UV dose delivered in joules per square centimeter (**Figs 3, 4**). Using a nonlinear regression model, the sunscreen with high UVA absorption revealed an IPF of 50, based on SFT increase. This value is more than three times the labeled SPF of 15. Clinical scoring resulted in a lower IPF value of 37, which is still more than twice the labeled SPF. The low UVA absorption sunscreen's IPF was equal to its labeled SPF of 15 based on SFT increase, and to 11 based on clinical scoring. To illustrate the immune protection benefit of high UVA protection on SFT, hypothetical dose–response curves were generated from the regression model of the immune responses of the unprotected subjects assuming that IPF was twice the SPF (i.e., 30) and three times the SPF (i.e., 45) (**Fig 5**). The resultant curves fall below the actual dose–response curve of product-A-protected subjects, graphically demonstrating and validating that the IPF of the high UVA absorption sunscreen is indeed more than twice and may be three times its SPF.

DISCUSSION

Skin cancer is the most common type of malignancy affecting white populations worldwide, and its incidence continues to increase in alarming proportions (Diepgen and Mahler, 2002). Because of the crucial role of immune suppression in the process of cutaneous carcinogenesis (Ullrich, 2002), protection against

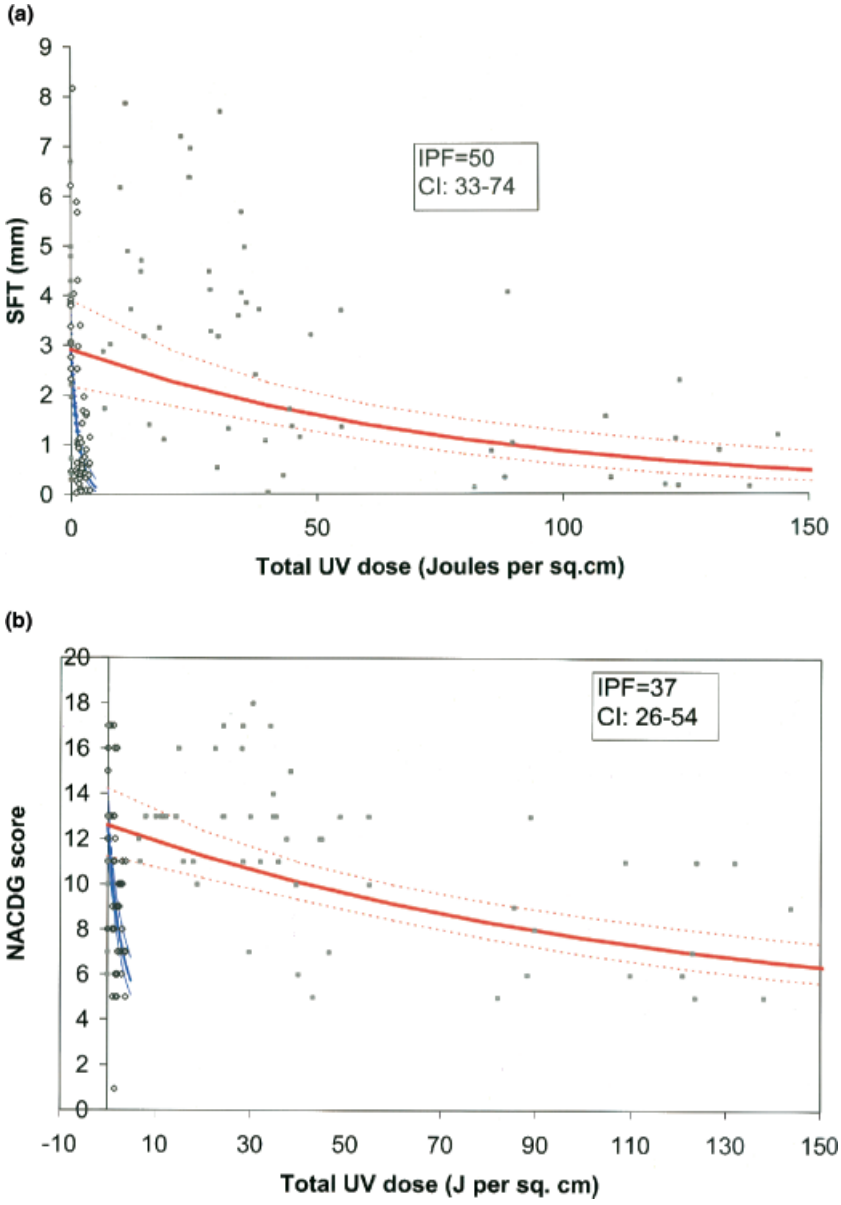

Figure 3. CHS responses via SFT increase (*a*) and NACDG score (*b*) were plotted against total UV dose delivered for the high UVA absorption sunscreen. Nonlinear regression analysis revealed higher IPF compared to the low UVA absorption sunscreen **(Fig 4)**. Sunscreen-treated individuals are represented by (■) and nontreated ones by (◇). Estimated IPF and associated confidence intervals are indicated in *boxes*. The UVR dose–response curves for suppression of CHS are represented in *blue* for nontreated according to the equation $y = \exp(a + b \times \text{dose})$ and in *red* for sunscreen-treated according to the equation $y = \exp(a + b \times \text{dose/IPF})$, with their confidence interval limits (*dashed lines*).

UV-induced immunosuppression has been the object of numerous research studies performed both in animal and human models (Bestak *et al*, 1995; Whitmore and Morrison, 1995; Damian *et al*, 1997; Moyal *et al*, 1997; Roberts and Beasley, 1997a; 1997b; Serre *et al*, 1997). Indeed, methods to prevent photoimmune suppression must be considered of high priority in the global campaign on skin cancer prevention. Whereas the immunosuppressive and carcinogenic potential of the more erythemogenic UVB spectrum is well established, less is known about UVA, even though UVA comprises 90%–95% of terrestrial UV radiation and is more penetrating (e.g., through glass windows) than UVB. More recent data suggest that the less erythemogenic UVA spectrum is much more involved in the process of immune suppression and carcinogenesis, including melanoma formation, than was originally appreciated (Setlow *et al*, 1993; Drobetsky *et al*, 1995; Bestak and Halliday, 1996; Kielbassa *et al*, 1997; Kvam and Tyrrell, 1997; Iwai *et al*, 1999; Nghiem *et al*, 2001; Wang *et al*, 2001). These data have led to the growing emphasis on broad-spectrum coverage and the necessity to evaluate a product's level of protection in the UVA range (UVA-PF), aside from its SPF.

Although it is known that sunscreens could protect from detrimental effects of UV other than erythema, our study demonstrates for the first time in human subjects that a broad-spectrum sunscreen with high UVA absorption could in fact afford significant protection against UV-induced CHS suppression, to a degree that greatly exceeds its capacity to prevent erythema (i.e., IPF > SPF). Because erythema remains the only well-defined biologic endpoint accepted by regulatory authorities for evaluating sunscreen efficacy (Federal Register, 1999), the SPF is often used in photobiologic studies for comparison with a novel entity such as IPF. Despite a lack of consensus regarding the standard model for determining IPF, our data clearly indicate that product A, with high absorption of both UVA and UVB, provided immune protection that is more than three times its SPF (**Fig 5**) and that such level of immune protection was definitely not observed with low filtration of UVA, despite equally high filtration of UVB, using product B, another broad-spectrum sunscreen with a similar SPF but with a lower UVA-PF. This confirms that a high UVA absorptive capacity is crucial in order for a product to optimally protect against immune suppression, which, in turn, indicates that UVA significantly contributes to local suppression of contact sensitivity induction in humans.

These findings obtained from *in vivo* human immune responses are supported by previous work focusing on UVA-induced

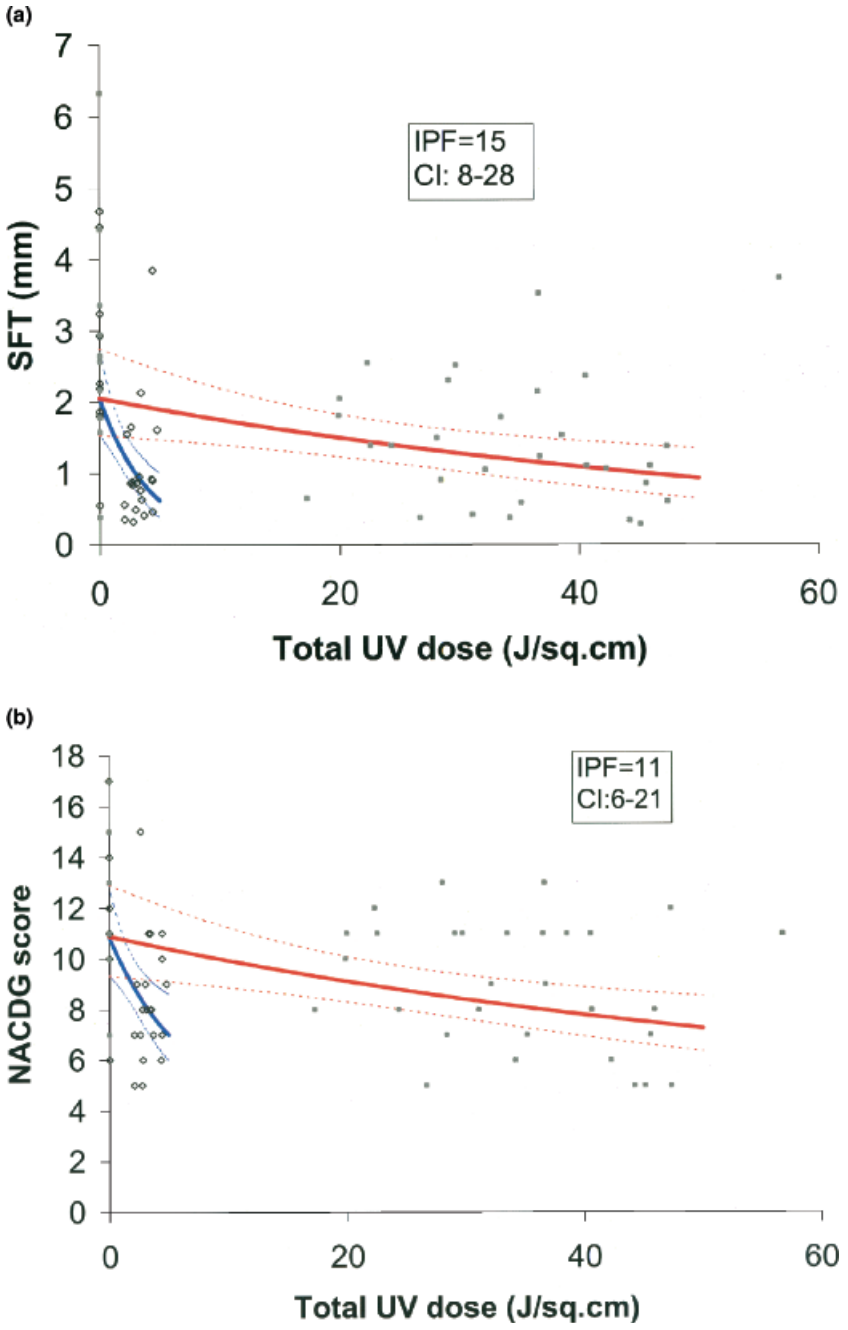

Figure 4. CHS responses via SFT increase (*a*) and NACDG score (*b*) were plotted against total UV dose delivered for the low UVA absorption sunscreen. Nonlinear regression analysis revealed higher IPF for the high UVA absorption sunscreen **(Fig 3)** compared to the low UVA absorption sunscreen. Sunscreen-treated individuals are represented by (■) and nontreated ones are represented by (◇). Estimated IPF and associated confidence intervals are indicated in *boxes*. The UVR dose–response curves for suppression of CHS are represented in blue for nontreated according to the equation $y = \exp(a + b \times \text{dose})$ and in *red* for sunscreen-treated according to the equation $y = \exp(a + b \times \text{dose/IPF})$, with their confidence interval limits (*dashed lines*).

changes within cells and cellular components that affect immunologic responsiveness. DNA is considered a major molecular target of UV radiation, in the induction of immune suppression. DNA damage in the form of pyrimidine dimers has been observed in human skin after UVA irradiation at a dose of 1 MED (Burren *et al*, 1998). Antigen presentation is altered by UVA (5–20 J per cm^2) via suppression of costimulatory molecule expression on Langerhans cells *in vitro* (Iwai *et al*, 1999). The same group of researchers likewise showed in mice that UVA (130 J per cm^2) causes suppression of lymph node cell proliferation in response to trinitrochlorobenzene. These effects were prevented by glutathione application, suggesting involvement of reactive oxygen species. Indeed, oxidative damage, another mechanism associated with photoimmune suppression, has been found to occur most efficiently within the UVA spectrum (Kielbassa *et al*, 1997; Kvam and Tyrrell, 1997). Dumay *et al* (2001) found that *in vivo* exposure of human skin to UVA1 (30 and 60 J per cm^2) results in decreased allogeneic CD4+ T cell proliferation. This was partially prevented by application of sunscreen containing 7% octocrylene and 3% butylmethoxydibenzoylmethane; however, no data on actual *in vivo* human responsiveness was provided. Furthermore, the inability to fully protect against UV-induced suppression of *in vitro* antigen-presenting cell function was attributed to the product's low UVA-PF (3 ± 0.2).

Epidemiologic studies on sunscreen use and the occurrence of melanoma have shown an increased incidence of this cancer among individuals who used sunscreens (Garland *et al*, 1993; Autier *et al*, 1995), although there is much controversy in the literature regarding such correlation. Our results suggest that the lack of adequate UVA filters in the vast majority of sunscreens marketed in the 1970s and 1980s permitted the immunosuppressive and other harmful biologic effects of UVA to take place but prevented

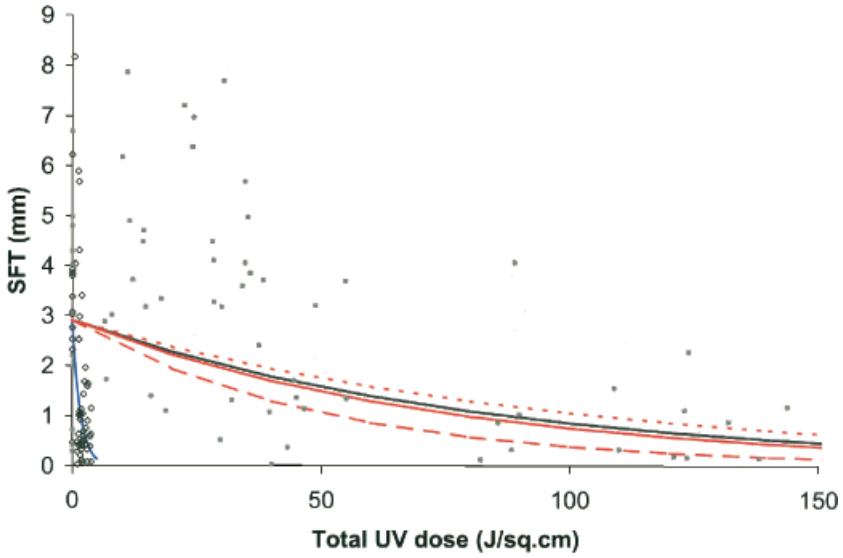

Figure 5. Hypothetical dose–response curves using nonlinear regression were constructed based on the SFT increase of unprotected, exposed subjects (blue line), assuming the IPF values were twice (long, discontinuous red lines), three times (continuous red line), and four times (short, discontinuous red lines) the labeled SPF. The actual curve for the CHS response of subjects protected by the high UVA absorption sunscreen is also plotted (black line). This illustrates that the IPF of the product is probably more than three times its SPF. Individual responses are represented by (■) for UVR + sunscreen and by (◇) for UV only.

UVB-induced changes, such as erythema, that provide warning that excessive exposure has occurred, thereby favoring melanoma development.

In summary, this study has shown that in human subjects UVA contributes significantly to SSR-induced immune suppression and the use of a broad-spectrum sunscreen with a sufficiently high UVA-PF results in protection against SSR-induced CHS suppression to a degree that exceeds the product's capacity to prevent erythema, and at a level that is significantly greater than the immune protection obtained when a product of equal SPF but a much lower UVA-PF was used. These results definitively demonstrate the etiologic role of UVA in immune suppression and confirm the growing evidence regarding the role of UVA in carcinogenesis. In the global attempt to promote sun protective measures and prevent skin cancer, it is critical to continually educate the public regarding the risks of excessive exposure to UVA (e.g., tanning beds), and to emphasize the need to use broad-spectrum sunscreens with adequate levels of UVA protection if sun avoidance is not possible.

This study was supported by L'Oréal Recherche, Clichy, France.

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